

- 1 -

67428/001.609

Claims

1. A method of detecting a plurality of different  
5 target nucleotide sequences present in a single sample,  
wherein said target sequences are detected at the same,  
or substantially the same, time and the method of  
detecting each nucleotide sequence in a nucleic acid  
molecule comprises:

10 (a) binding of an oligonucleotide probe to said  
nucleic acid molecule;

(b) selective labelling of the bound  
oligonucleotide probe in the presence of said target  
nucleotide sequence;

15 (c) hybridisation of the labelled oligonucleotide  
to a complementary sequence; and

(d) subsequent detection of the label.

2. A method as claimed in claim 1 wherein the  
20 complementary sequence of (c) is fully complementary to  
the oligonucleotide probe.

3. A method as claimed in claim 1 or claim 2 wherein  
the oligonucleotide probe is 20 to 30 nucleotides in  
25 length.

4. A method as claimed in any of the preceding claims  
wherein the oligonucleotide probe is labelled by  
incorporation of a labelled nucleotide.

30 5. A method as claimed in claim 4 wherein the labelled  
nucleotide is a labelled dideoxynucleotide.

35 6. A method as claimed in claim 4 or 5 wherein  
selective labelling takes place in the presence of one  
or more labelled dideoxynucleotides and one or more  
unlabelled dideoxynucleotides.

- 2 -

7. A method as claimed in claim 6 wherein selective labelling takes place in the presence of one labelled dideoxynucleotide and three unlabelled dideoxynucleotides.

5

8. A method as claimed in any of the preceding claims wherein the oligonucleotide probe is designed with one or more mismatches at the 3'-end to non-target nucleotide sequences.

10

9. A method as claimed in any one of the preceding claims wherein a plurality of labelling steps are performed consecutively.

15

10. A method as claimed in any of the preceding claims wherein the sequence complementary to the labelled oligonucleotide is immobilised on a solid support.

20

11. A method as claimed in claim 10 wherein the solid support is a membrane strip or nucleic acid chip.

25

12. A method as claimed in any of the preceding claims wherein steps (a) to (d) are preceded by amplification of the nucleic acid molecule which contains the target sequence.

13. A method as claimed in claim 12 wherein the nucleic acid molecule which contains the target sequence is co-amplified with a competitor nucleic acid molecule.

30

14. A method as claimed in claim 13 wherein the competitor molecule comprises a recognition sequence which is complementary to a competitor oligonucleotide probe.

35

15. A method as claimed in claim 14 wherein the competitor oligonucleotide probe is selectively labelled

- 3 -

after hybridisation to the competitor molecule.

16. A method as claimed in claim 15 which additionally comprises hybridisation of the labelled competitor  
5 oligonucleotide to a complementary sequence and subsequent detection of the label.

17. A method as claimed in any of the preceding claims wherein the sequences which are complementary to the  
10 oligonucleotide probes are immobilised on a solid support in discrete, pre-determined positions.

18. A method of determining the amount of a target nucleotide sequence or the number of cells containing a  
15 target nucleotide sequence, which comprises a detection method as claimed in any one of claims 1 to 17.

19. A method of detecting the presence of bacteria in a sample which comprises a method as claimed in any one of  
20 claims 1 to 17.

20. A method as claimed in claim 19 wherein the bacteria are cyanobacteria.

25 21. A kit, for carrying out a method as claimed in any of the preceding claims which comprises:

(a) oligonucleotide probes capable of binding to target nucleic acid molecules containing target nucleotide sequences;

30 (b) means for selective labelling of the oligonucleotide probes; and

(c) nucleotide sequences complementary to the oligonucleotide probes, preferably immobilised on a solid support.